Introgression of novel disease resistance genes from *Miscanthus* into energycane (Final Report)

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COLLABORATORS:

Keiji Motomura	University of the Ryukyus	Japan
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PROJECT DURATION:
08/15/2016 to 08/14/2023

DOE/OFFICE OF SCIENCE: OFFICE OF BIOLOGICAL AND ENVIRONMENTAL RESEARCH

Abstract:
Sugarcane (*Saccharum*) is among the world's leading bioenergy crops. However, modern sugarcane cultivars are derived from a relatively small set of founder genotypes, which has contributed to cultivar susceptibility to diseases. *Miscanthus* is a close relative of sugarcane that is genetically diverse and a potential source of genes for improving sugarcane. We found that *Miscanthus* is a source of resistance to four major diseases of sugarcane and we crossed these disease-resistance genes into a predominantly sugarcane genetic background (BC1 generation). Additionally, we found that *Miscanthus* could also confer genes for chilling-tolerant photosynthesis to sugarcane, which would be highly advantageous for production of energycane in subtropical environments, like the southern coastal plain of the US. Using advanced modeling techniques, we found that standard marker-assisted selection could be effective for breeding resistance conferred by a small number of genes each with large effect (i.e. vertical resistance), but to breed for many genes each of small effect (i.e. horizontal resistance), genomic selection would be the better strategy. Lastly, we learned that *Miscanthus* can be induced to flower sooner by giving the plants short days (long nights) but that the ideal day length for a given accession depends on its adaptation to its latitude of origin, with tropical accessions requiring shorter days to flower than accessions from high latitudes. This information will facilitate plant breeders' ability to make crosses between *Miscanthus* and sugarcane, and enable greater use of *Miscanthus* as a genetic resource to improve sugarcane.

Project objectives:

A. In miscane BC_1 populations (sugarcane x (sugarcane x *Miscanthus*)), identify molecular markers associated with novel genes from *Miscanthus* that confer resistance to at least two out of four of the following economically important diseases of sugarcane: ratoon stunt, yellow leaf, orange rust, and smut.

B. Compare effectiveness of different molecular marker analysis methods for selecting disease resistance alleles in miscane backcross populations. In particular, compare the pseudo-testcross QTL mapping strategy (the gold standard for sugarcane) with genomic selection.

C. Screen germplasm collections of *M. sinensis* and *M. sacchariflorus* for resistance to ratoon stunt, yellow leaf, orange rust, and smut to confirm that *Miscanthus* is uniformly immune to these diseases as prior data suggest or to quantify genetic variation for resistance if not all accessions are resistant.

Accomplishments, and impacts:

<u>Disease screens of *Miscanthus* and miscane germplasm</u>:
A panel of 72 genotypes was successfully screened at Canal Point, FL by collaborator Sood for four key diseases of sugarcane (Table 1). The panel included a mini-core collection of 31 *M. sacchariflorus* (Msa), a mini-core collection of 35 *M. sinensis* (Msi), and 6 miscanes. The *Miscanthus* accessions were selected to represent each of the Msa and Msi genetic groups that we identified in previous DOE-funded studies. All or nearly all *Miscanthus* entries tested were highly resistant to orange rust, ratoon stunt, and smut (Table 1). For sugarcane yellow leaf, 58% of *Miscanthus* entries were resistant, with resistance more frequent in *M. sinensis* than *M. sacchariflorus*. Thus, the results of the replicated disease screens, confirmed our hypothesis that *Miscanthus* is an excellent source of alleles for resistance to the four most important diseases of sugarcane.

Table 1. Percentage of *M. sacchariflorus* (Msa), *M. sinensis* (Msi), and miscane genotypes that were resistant to four diseases of sugarcane based on evaluations at Canal Point, FL.

Diseases	**Replicates**	**Positive control**	**Negative control**	**Msa (n=31)**	**Msi (n=35)**	**Miscanes (n=6)**
Orange rust	Four pots	CP96-1252	CP89-2143	100%	97%	83%
Ratoon stunt	Three shoots/pot (4 pots, total 12 shoots)	CP72-2086	CP72-1210	97%	97%	67%
Smut	Three shoots/pot (4 pots, total 12 shoots)	CP72-2086	CP78-1628/CP57-603	100%	100%	83%
Yellow leaf	Three leaves/pot (4 pots, total 12 leaves)	CP57-603	Virus free CP80-1743	45%	69%	50%

Of the six miscane genotypes tested, one was fully resistant to all four diseases, six were fully resistant to orange rust and smut, four were resistant to ratoon stunt, and three were resistant to sugarcane yellow leaf virus (SCYLV). Thus, introgression of disease-resistance from *Miscanthus* into sugarcane is feasible.

Backcross population development and disease screening:
Crosses between miscanes and sugarcane (i.e. backcrosses to cane) were made in IL and FL. From the crosses made at IL in the greenhouse, seed was sent to ARS in FL for germination and backcross progeny were obtained. Most of the seedlings produced were derived from the miscane accession that we had found to be resistant to all four of the key diseases of sugarcane (orange rust, ratoon stunt, smut, and SCYLV). The backcross progenies were screened for disease-resistance and were found to segregate, confirming that it is possible to introgress genes for resistance to key diseases from *Miscanthus* into energycane. Moreover, these selections are being integrated into USDA's applied cane breeding program at Canal Point.

Model simulations to compare genomic selection with marker-assisted selection:
Co-PI Lipka led the successful development of a pipeline that simulated traits with various genetic architectures in an F_1 population, and then assess the ability of marker-assisted selection (MAS) and genomic selection (GS) to predict trait values of individuals in a simulated backcross generation. This pipeline is described in detail in Figure 1. A postdoctoral researcher, Dr. Marcus Olatoye, was trained by Dr Lipka and this pipeline to simulate traits. A summary of the performance of MAS and GS for a series of simulated traits simulated in a *M. sinensis* F_1 population are presented in Figures 2 and 3. Briefly, these results show that MAS yields higher prediction accuracies for simpler traits controlled by a smaller number of quantitative trait nucleotides (QTNs), whereas GS yields higher prediction accuracies for traits controlled by a larger number of QTNs. As the number of simulated QTN was increased, all GS models that were evaluated tended to outperform MAS, select more phenotypically optimal F1 individuals, and accurately predict simulated trait values in subsequent BC1 generations. We therefore concluded that GS is preferable to MAS for introgressing genetic sources of horizontal disease resistance from *Miscanthus* to energycane, while MAS remains a suitable option for introgressing vertical disease resistance.

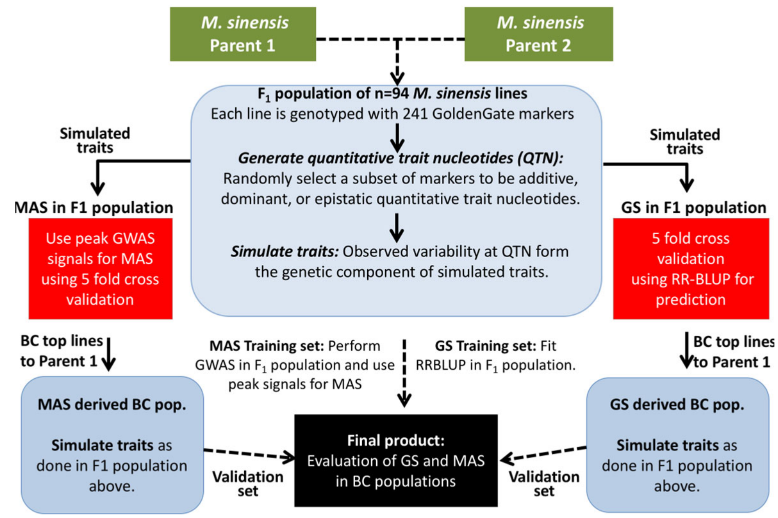

Figure 1. Simulation pipeline developed to assess the prediction accuracy of marker-assisted selection (MAS) and genomic selection (GS) for traits simulated in an F_1 population of *Miscanthus sinensis* parents. The individuals with optimal predicted trait values from MAS and GS are independently backcrossed to Parent 1; the trait is then simulated in the backcross (BC) populations to assess prediction accuracy from MAS and GS models trained in the F_1 population.

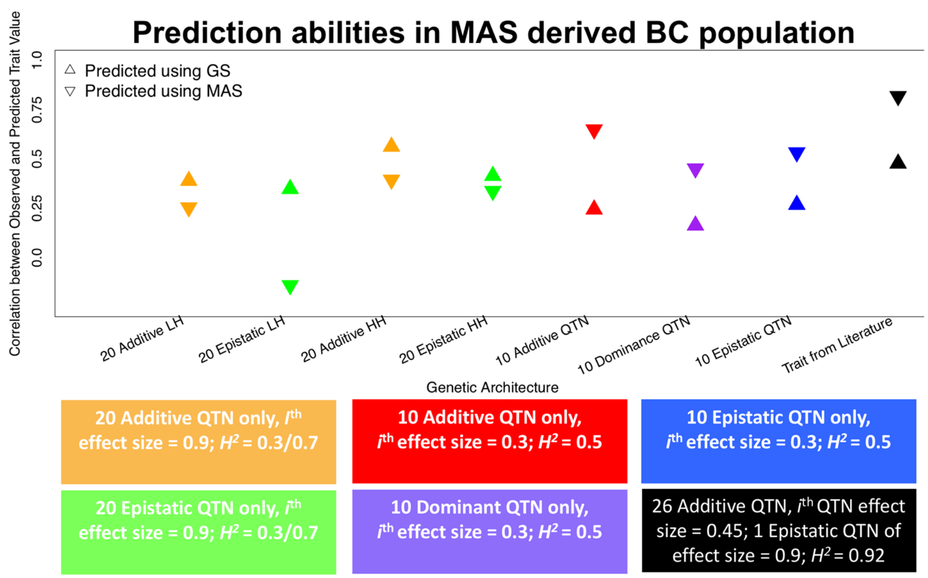

Figure 2. Results of genomic selection (GS) and marker-assisted selection (MAS) prediction accuracies in the MAS-derived backcross population, where traits with various genetic architectures are simulated in the pipeline illustrated in Figure 1. The X-axis depicts the genetic architectures that were simulated and the Y-axis depicts the prediction accuracy of GS (right side up triangle) and MA (upside down triangle). The various colors of the triangles correspond to the simulated genetic architectures, each of which are described in the rectangles with the same color below the graph. HH = high heritability (H^2 = 0.7), LH = low heritability (H^2 = 0.3), QTN = quantitative trait nucleotides. In general, these results suggest that GS tends to outperform MAS for traits with a larger number of underlying QTNs.

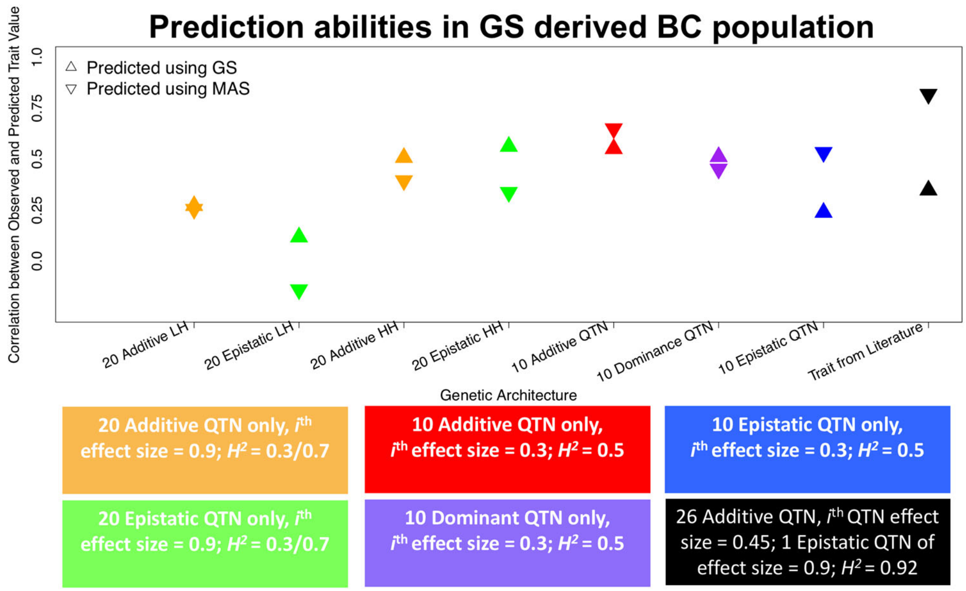

Figure 3. Results of genomic selection (GS) and marker-assisted selection (MAS) prediction accuracies in the GS-derived backcross population, where traits with various genetic architectures are simulated in the pipeline illustrated in Figure 1. The X-axis depicts the genetic architectures that were simulated and the Y-axis depicts the prediction accuracy of GS (right side up triangle) and MA (upside down triangle). The various colors of the triangles correspond to the simulated genetic architectures, each of which are described in the rectangles with the same color below the graph. HH = high heritability (H^2 = 0.7), LH = low heritability (H^2 = 0.3), QTN = quantitative trait nucleotides. In general, these results suggest that GS outperforms MAS for traits with a larger number of underlying QTNs.

Differential expression of genes for disease-response and low temperature between a miscane and its *Miscanthus* and cane parents:
Comparisons were made by Co-PI Yamada between sugarcane ‘KY-06-139’, *Miscanthus sacchariflorus* ‘Miyakonojo’ and their miscane progeny ‘JM14-72’ and ‘JM-14-88’. A comparison of candidate disease-response genes under unchallenged conditions revealed that expression in the miscane progeny was in some cases upregulated relative to both parents and in other cases downregulated. For low temperature tolerance, Yamada observed photosynthetic efficiency of the miscane progeny during the cool spring and autumn was similar to *Miscanthus* and greater than in the sugarcane parent, whereas in the summer all of the entries had similarly high levels of photosynthesis (Fig. 4). Similar responses were observed under controlled environment conditions, when these genotypes were given a low temperature treatment of 15°C /10°C (10h light/14h dark) for three weeks. Gene expression analyses of plants subjected to low temperature provided insights into similarities and differences in how sugarcane, *Miscanthus* and their miscane progeny responded (Fig. 5).

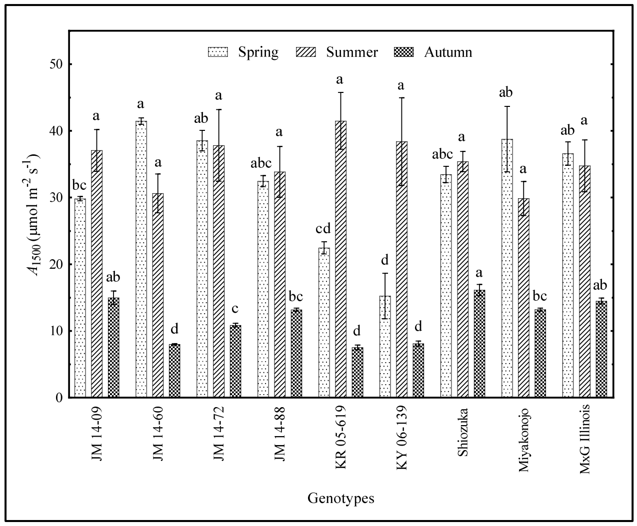

Figure 4. Seasonal variation in the CO_2 assimilation rate (mean ± SE) of genotypes *in situ* at solar noon ± 2 h. Different letters indicate significant difference (n = 3) between genotypes within each season.

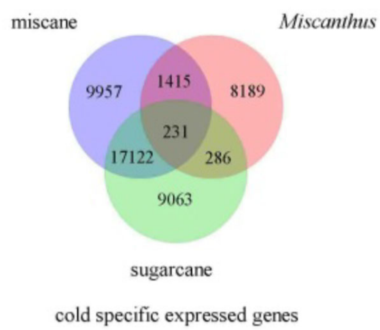

Figure 5. Venn diagram of cold specific gene expression in sugarcane, *Miscanthus* and their miscane progeny.

<u>Understanding the biology of flowering time in *Miscanthus* and sugarcane to facilitate timing of intergeneric crosses</u>:
PI Sacks conducted a series of experiments in greenhouses at Urbana, IL and in growth chambers to study the effect of day length on flowering of both *Miscanthus* and sugarcane. Both *Miscanthus* and sugarcane were found to be short-day plants but response to day length in *Miscanthus* is facultative and depended greatly on the latitude of origin of the accession. with 12.5 hours of day length in a growth chamber, *M. sinensis* from low latitudes would flower but accessions from high latitudes would transition to a dormancy response of very short internodes. In contrast, at 15 hours of day length, *M. sinensis* from high latitudes would flower but accessions from low latitudes remained vegetative. Sugarcanes could be induced to flower in a greenhouse at Urbana, IL (~40 °N) by using supplemental lighting, starting in mid-September when natural day length was 12.5 hours, to reduce the rate at which days shortened during the autumn and winter to 1 min d^{-1}, which allowed us to synchronize the flowering of some sugarcane genotypes with *Miscanthus* genotypes.

In a complementary study, co-PI Yamada evaluated a mini-core collection of *M. sinensis* for sequence diversity of *Ghd8*, a transcription factor encoding a HAP3/NF-YB DNA-binding domain, which has been identified as a major quantitative trait locus in rice, with pleiotropic effects on grain yield, heading date and plant height. We identified two homoeologous loci, *MsiGhd8A* located on chromosome 13 and *MsiGhd8B* on chromosome 7, with one on each of this paleo-allotetraploid species' subgenomes. A total of 46 alleles and 28 predicted protein sequence types were identified in 12 wild-collected accessions. S everal variants of *MsiGhd8* showed a geographic and latitudinal distribution. Quantitative real-time PCR revealed that *MsiGhd8* expressed under both long days and short days, and *MsiGhd8B* showed a significantly higher expression than *MsiGhd8A*. The comparison between flowering time and gene expression indicated that *MsiGhd8B* affected flowering time in response to day length for some accessions. This study provided insight into the conserved function of Ghd8 in the Poaceae, and is an important initial step in elucidating the flowering regulatory network of *Miscanthus*.

Participants & other collaborating organizations:
Dr. Alex Lipka, a co-investigator on the project and statistician, provided leadership on modeling of genomic selection.

Dr. Marcus Olatoye began work as a post-doctoral researcher with CoPI Lipka in January 2018.

Dr. Nick Labonte worked as a post-doctoral researcher with PI Sacks starting in February 2018 and left in 2020 for permanent position with USDA Forest Service.

Dr. Jack Comstock, a co-investigator on the project, unexpectedly retired from USDA early in 2017 for personal reasons. However, Dr. Comstock played a key role in coordinating the initial experiments and he continued to provide intellectual input to the project via his current affiliation with the University of Florida.

With Dr. Comstock's retirement, Dr. Vanessa S. Gordon, a co-investigator on the project, assumed responsibility for overall coordination of the project's activities at Canal Point for a time but subsequently left ARS for personal reasons.

Dr. Sushma Sood, a plant pathologist who worked closely with Dr Comstock, took the leading role in conducting the disease screens and ultimately for all of the project's activities at Canal Point.

Dr. Per McCord, a Research Geneticist at the USDA ARS Sugarcane Field Station collaborated with our project by making crosses between miscanes and canes, and by providing guidance on how to time flowering of canes.

Dr. Shailendra Sharma, who contributed to the molecular identification of miscane hybrids in the U.S., left UIUC for a permanent position based at Ch. Charan Singh University in India. Dr. Sharma will continue to collaborate from India with this project but without funding from the project.

Dr. Ken-Taro Sekine, a plant pathologist and Professor at the University of the Ryukyus, collaborated with us on conducting disease-screens in Okinawa.

Dr. Toshihiko Yamada, a Professor at Hokkaido University and co-investigator on the project, produced miscanes from controlled crosses with northern-adapted *Miscanthus* and studied these.

Deliverables:

Publications:

1) Sood, S., R. Bruyere, N. LaBonte , and E.J. Sacks. (In preparation). Introgression of genes for resistance to diseases from *Miscanthus* into energycane.
2) Sood, S., R. Bruyere, N. LaBonte, J.C. Comstock, A.E. Lipka, V. Gordon, B.K. Ghimire, K. Heo, X. Jin, J. Peng, J.H. Yoo, C.Y. Yu, H. Zhao, T. Yamada, K. Motomura, and E.J. Sacks. (In preparation). *Miscanthus* is a potential source of resistance to diseases of sugarcane.
3) Sharma, S., Y. Terashima, C. Hervouet, K. Glowacka, L.V. Clark, V.S. Gordon, T. Abbott, J. Comstock, R. Ming, A. D'Hont, T. Yamada, and E.J. Sacks. (In preparation). Phylogentic relationships within and between *Miscanthus* and *Saccharum*: Implications of natural and artificial miscanes for the *Saccharum* complex hypothesis.
4) Dong, H., L.V. Clark, X. Jin, K. Anzoua, L. Bagmet, P. Chebukin, E. Dzyubenko, N. Dzyubenko, B.K. Ghimire, K. Heo, D.A. Johnson, H. Nagano, A. Sabitov, J. Peng, T. Yamada, J.H. Yoo, C.Y. Yu, H. Zhao, S.P. Long, and E.J. Sacks. 2021. Managing flowering time in *Miscanthus* and sugarcane to facilitate intra- and intergeneric crosses. PLoS ONE 16:e0240390 https://doi.org/10.1371/journal.pone.0240390.
5) Guo, Z., M. Xu, H. Nagano, L.V. Clark, E.J. Sacks, and T. Yamada. 2021. Characterization of the *Ghd8* flowering time gene in a mini-core collection of *Miscanthus sinensis*. Genes 12:288 https://doi.org/10.3390/genes12020288.
6) Kar, S., T.Y. Weng, T. Nakashima, A. Villanueva-Morales, J.R. Stewart, E.J. Sacks, Y. Terajima, and T. Yamada. 2020. Field performance of *Saccharum* × *Miscanthus* intergeneric hybrids (Miscanes) under cool climatic conditions of northern Japan. Bioenerg. Res. 13:132–146 https://doi.org/10.1007/s12155-019-10066-x.
7) Olatoye, M.O., L.V. Clark, N.R. Labonte, H. Dong, M.S. Dwiyanti, K.G. Anzoua, J.E. Brummer, B.K. Ghimire, E. Dzyubenko, N. Dzyubenko, L. Bagmet, A. Sabitov, P. Chebukin, K. Głowacka, K. Heo, X. Jin, H. Nagano, J. Peng, C.Y. Yu, J.H. Yoo, H. Zhao, S.P. Long, T. Yamada, E.J. Sacks, and A.E. Lipka, 2020. Training Population Optimization for Genomic Selection in *Miscanthus*. G3: Genes, Genomes, Genetics 10:2465–2476 https://doi.org/10.1534/g3.120.401402.
8) Olatoye, M.O., L.V. Clark, J. Wang, X, Yang, T. Yamada, E.J. Sacks, and A.E. Lipka. 2019. Evaluation of genomic selection and marker-assisted selection in *Miscanthus* and energycane. Mol. Breeding 39:171 https://doi.org/10.1007/s11032-019-1081-5.
9) Kar, S., N. Zhang, T. Nakashima, A Villanueva-Morales, J.R. Stewart, E.J. Sacks, Y. Terajima, and T. Yamada. 2019. *Saccharum* × *Miscanthus* intergeneric hybrids (miscanes) exhibit greater chilling tolerance of C_4 photosynthesis and postchilling recovery than sugarcane (*Saccharum* spp. hybrids). GCB Bioenergy 11:1318-1333 https://doi.org/10.1111/gcbb.12632.
10) Poster presented at Plant and Animal Genome Conference in January 2018:
    P0228 Evaluation of Marker-Assisted Selection and Genomic Selection in Polyploid Backcross Populations. Alexander E. Lipka, Lindsay V. Clark, Xiping Yang, Jianping Wang, D.K. Lee, Claire Liu, Keiji Motomura, Sushma Sood, Vanessa S. Gordon, Toshihiko Yamada and Erik J. Sacks. https://pag.confex.com/pag/xxvi/meetingapp.cgi/Paper/30005.

Training:

Trainee	Under-graduate	Graduate	Post-doctoral	Technical	Institution
Maiko Ohta				X	HU
Suraj Kar		X			HU
Zhihui Guo		X			HU
Kossonou Anzoua			X		HU
Na Zhang			X		HU
Tzu-Ya Weng		X			HU
Kiko Eguchi		X			HU
Lindsay Clark			X		UIUC
Hongxu Dong		X			UIUC
Nick Labonte			X		UIUC
Colten Maertens				X	UIUC
Marcus Olatoye			X		UIUC
Shailendra Sharma			X		UIUC

Note on the pandemic:

The pandemic disrupted the work of this project considerably. During this time, most of the researchers on this project were required to work from home. This necessitated no-cost extensions, which as indicated by the substantial number of publications resulting from this project, were very productive.